HHS Public Access

Author manuscript

J Immunother. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

J Immunother. 2018 April ; 41(3): 151–157. doi:10.1097/CJI.0000000000000220.

A Dose-Escalation Study of Recombinant Human Interleukin-18 in Combination with Ofatumumab after Autologous Peripheral Blood Stem Cell Transplantation for Lymphoma

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Summary

Interleukin-18 (IL-18) is an immunostimulatory cytokine that augments antibody-dependent cellular cytotoxicity mediated by human NK cells against antibody-coated lymphoma cells in vitro and that has antitumor activity in animal models. Ofatumumab is a CD20 monoclonal antibody with activity against human B cell lymphomas. A phase I study of recombinant human (rh) IL-18 given with ofatumumab was undertaken in patients with CD20+ lymphoma who had undergone high-dose chemotherapy and autologous peripheral blood stem cell transplantation. Cohorts of 3 patients were given intravenous infusions of ofatumumab 1000 mg weekly for 4 weeks with escalating doses of rhIL-18 as a intravenous infusion weekly for 8 consecutive weeks. Nine male patients with CD20+ lymphomas were given ofatumumab in combination with rhIL-18 at doses of 3, 10, and 30 μg/kg. No unexpected or dose-limiting toxicities were observed. The mean reduction from pre-dose levels in the number of peripheral blood NK cells after the first rhIL-18 infusion was 91%, 96%, and 97% for the 3, 10, and 30 μg/kg cohorts, respectively. Serum concentrations of interferon (IFN)-γ and chemokines transiently increased following IL-18 dosing. rhIL-18 can be given in biologically active doses by weekly infusions in combination with ofatumumab after PBSCT to patients with lymphoma. A maximum tolerated dose of rhIL-18 plus ofatumumab was not determined. Further studies of rhIL-18 and CD20 monoclonal antibodies in B cell malignancies are warranted.

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Conflicts of Interest

All other authors have declared that there are no financial conflicts of interest in regard to this work.

DISCLOSURE OF OFF-LABEL USE / UNAPPROVED DRUGS

Ofatumumab is not approved by the FDA for treatment of lymphoma. SB-485232 (iboctadekin) is an investigational drug and not approved by the FDA for any indication.

Keywords

Ofatumumab; CD20; IL-18; IFN-γ; lymphoma

Introduction

Non-Hodgkin lymphomas (NHL) are the most common of the hematologic malignancies (1). Despite substantial improvement in treatment of B cell lymphomas since the advent of rituximab, approximately 30% of patients with diffuse large B cell lymphoma (DLBCL) and virtually all patients with advanced stage indolent lymphoma will relapse after treatment with rituximab-based regimens. High-dose therapy and autologous peripheral blood stem cell transplantation (PBSCT) is the treatment of choice for eligible patients with relapsed DLBCL that is responsive to salvage chemotherapy (2–5). PBSCT is also a reasonable treatment option for patient with relapsed indolent lymphoma. The overwhelming cause of treatment failure after PBSCT is progression of lymphoma. Substantial improvement in the outcome of PBSCT for NHL will require strategies that can reduce the risk of relapse after transplantation. One approach is post-transplant immunotherapy to try to eliminate chemotherapy-resistant tumor cells (6). The state of minimal residual disease that can occur post-transplant may be a particularly promising setting for cancer immunotherapy, as the latter is most effective against a relatively small tumor burden.

Our previous studies indicate that a major obstacle to successful cancer immunotherapy after PBSCT is acquired STAT4 deficiency. We have found that IFN-γ production during IL-12 therapy is markedly defective in cancer patients who have undergone high-dose chemotherapy and PBSCT (7, 8). Defective IFN-γ production in this setting is due to a profound and selective deficiency in STAT4 (7, 8). STAT4 deficiency in the immune system of cancer patients is expected to impair clinical immunotherapy that requires Th1 immune responses and optimal production of IFN-γ. Preclinical studies indicate that IFN-γ plays a pivotal role in the cellular immune response to tumors (9–11). Therefore, development of strategies to circumvent STAT4 deficiency is critical for effective cancer immunotherapy after PBSCT. STAT4 is not known to participate in the signaling pathways required for IFN-γ production in response to IL-18 or after stimulation of NK cells via ligation of CD16 (12, 13). It is therefore rational to combine rhIL-18 with CD20 monoclonal antibodies for the immunotherapy of lymphoma after PBSCT.

IL-18 is an immunostimulatory cytokine that regulates both innate and adaptive immune responses (14, 15). Administration of IL-18 to cancer patients is safe and causes the in vivo activation of human NK cells (16, 17). A phase I trial of rhIL-18 plus rituximab has confirmed the safety and immunologic activity of this combination in patients with relapsed and refractory lymphomas (18). Administration of rhIL-18 could promote antitumor immune responses by augmenting ADCC of NK cells and monocytes, stimulating production of IFN-γ, MIG, and IP-10, enhancing differentiation of Th1 cells, and facilitating recruitment of effector cells to tumor sites. Other cytokines, including IL-2, IL-12, and GM-CSF, have been given in combination with rituximab to treat patients with lymphoma (19–23). However, IL-18 may be preferable to these cytokines for combined immunotherapy with CD20

monoclonal antibodies. IL-2 and IL-12 can augment antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells, but do not appear to significantly enhance ADCC by monocytes or macrophages (24–27). In contrast, GM-CSF preferentially stimulates ADCC by monocyte/macrophages and has little effect on NK cell cytolytic activity (25, 27, 28). IL-18 can strongly activate both NK cells and monocyte/macrophages (14, 15, 29), and hence might be more potent than IL-2, IL-12, or GM-CSF in enhancing ADCC against antibody-sensitized lymphoma cells. Furthermore, administration of IL-2 leads to the in vivo expansion of CD25+ CD4+ regulatory T cells, which can inhibit IFN-γ production and antitumor immune responses (30–32). Both standard dose and high dose chemotherapy cause an acquired STAT4 deficiency in lymphoma patients, which leads to impaired IL-12-induced immune responses (33–35). Therefore, IL-18 may prove more effective than IL-2 or IL-12 for cytokine-based immunotherapy of lymphoma.

Ofatumumab is a fully human IgG1 monoclonal antibody that binds to different epitope of CD20 than that recognized by rituximab (36). Ofatumumab can be safely given to patients with relapsed and refractory lymphoma (37). Compared to rituximab, ofatumumab mediates more potent ADCC and complement-dependent cytotoxicity against CD20+ lymphoma cells in vitro (38–41). Moreover, SCID mice bearing disseminated human lymphoma xenografts have superior survival after treatment with rhIL-18 plus ofatumumab compared to treatment with rhIL-18 plus rituximab (42); GlaxoSmithKline, unpublished data shared with the PI). We hypothesized that rhIL-18 and ofatumumab would evoke more effective antitumor immune responses than rhIL-18 plus rituximab in patients with lymphoma who have undergone PBSCT.

PATIENTS AND METHODS

Patient Selection

Eligible patients included adults (age ≥ 18 years) with histologically confirmed CD20+ B cell NHL who had undergone high-dose therapy and autologous PBSCT within 2–6 months prior to study enrollment. Patients were required to have ECOG performance status less than 2 as well as adequate renal, hepatic, and hematologic function. Patients were excluded if they had obvious clinical progression of lymphoma after PBSCT, were pregnant or breast-feeding, were seropositive for HIV or hepatitis B surface antigen, had active hepatitis C infection, known symptomatic or untreated leptomeningeal or brain metastases, or major uncontrolled co-morbid illnesses.

Study Design

The study was an open-label, non-randomized, dose-escalation phase I clinical trial (GlaxoSmithKline Clinical Study OFT116979; ClinicalTrials.gov identifier NCT01768338). The study protocol was approved by the Institutional Review Board at Indiana University Medical Center (Indianapolis, IN). Written informed consent was obtained from each patient prior to enrollment on study. Study drugs SB-485232 (iboctadekin), a rhIL-18 protein produced in *E. coli*, and ofatumumab were supplied by GlaxoSmithKline (Research Triangle Park, NC). After the clinical trial was initiated, responsibility for study drugs was transferred from GlaxoSmithKline to Novartis (East Hanover, NJ). The IND for iboctadekin and

ofatumumab was held by the principal investigator (MJR). Ofatumumab 1000 mg was given by intravenous infusion once per week for four consecutive weeks (on day 1 of weeks 1–4). rhIL-18 was given by intravenous infusion over 2 hours once per week for 8 consecutive weeks (on day 2 of weeks 1–8). During weeks 1–4 of study, the rhIL-18 infusions were started at least 24 hours after initiation of the preceding ofatumumab infusion.

Successive cohorts of 3 patients were planned to receive rhIL-18 in doses of 3, 10, 30, and potentially 100 μg/kg. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria Version 4.0. Dose-limiting toxicity was defined as any grade 3 or 4 toxicity observed during the first 6 weeks of treatment that was assessed to be related to study drug, excluding grade 3–4 lymphopenia and grade 3 nausea or vomiting that was responsive to supportive care measures.

Prospectively defined biomarker analyses were used to determine whether dose escalation should halt at the 30 μg/kg cohort or proceed to the 100 μg/kg cohort. Transient lymphopenia is commonly observed after the administration of immunostimulatory cytokines and is most likely due to in vivo activation of lymphocytes with their subsequent extravasation into tissue spaces and/or infiltration into tumors (7, 43, 44). Transient decrease in the absolute number of peripheral blood NK cells after rhIL-18 infusions was considered a biomarker for NK cell activation in vivo. Escalation to a 100 μg/kg dose cohort was to be undertaken only if an average decrease of less than 80% from baseline NK cell number was seen after the first infusion of rhIL-18 in the 30 μg/kg dose cohort.

Pharmacodynamic Studies

Blood samples were collected for analysis of leukocyte markers by flow cytometry prior to initiation of ofatumumab infusion on day 1 of week 1 and prior to and 4 hours after initiation of the rhIL-18 infusion on day 2 of weeks 1, 4, and 8 of study. Blood samples were collected for quantification of plasma IFN-γ and chemokines prior to initiation of ofatumumab infusion on day 1 of weeks 1–4 and prior to and 4 and 6 hours after initiation of rhIL-18 infusion on day 2 of weeks 1, 4, and 8 of study. Peripheral blood mononuclear cells (PBMCs) were isolated on a Ficoll-diatrizoate gradient from venous blood samples.

PBMCs were stained directly with fluorochrome-conjugated monoclonal antibodies, washed, fixed in 1% formaldehyde, and analyzed by flow cytometry as previously described (45) using an FACScan or FACScalibur instrument from Becton Dickinson (San Diego, CA). The absolute number of peripheral blood NK cells was calculated by multiplying the total lymphocyte count (derived from a routine complete blood count performed at the same time a blood sample was obtained for flow cytometry studies) by the percent of cells in a sample expressing CD56 in the absence of CD3 (derived from flow cytometric analysis).

Levels of IFN-γ, MIG (CXCL9), and IP-10 (CXCL10) protein in serum samples were measured using specific ELISA kits according to the manufacturer's (R & D Systems, Minneapolis, MN) instructions. The lower limit of detection for the IFN-γ ELISA was 8 pg/mL. For data analysis, samples with IFN-γ levels < 8 pg/mL were assigned a value of 8 pg/mL. The upper limit of linearity for IP-10 ELISA was 500 pg/mL. For data analysis, samples with IP-10 levels exceeding 500 pg/mL were assigned a value of 500 pg/mL.

Evaluation of Clinical Characteristics and Response

Lymphoma subtypes were designated using the WHO Classification (46). Patients designated to have primary refractory disease were those who failed to achieve complete response or who developed objective disease progression ≤ 3 months after receiving a first-line chemotherapy regimen. At the time of PBSCT, patients designated to have chemosensitive disease were those who achieved a complete or partial response after receiving salvage chemotherapy prior to PBSCT, whereas patients who failed to achieve a complete or partial response after salvage chemotherapy were designated to have chemorefractory disease. Radiographic evaluation of tumor responses was done as per standard practice and was not dictated by the study protocol. Tumor responses were assessed using International Workshop Criteria (47).

Statistical Analysis

Analysis of safety and efficacy data was descriptive in nature, with counts and percentages determined for categorical data and mean, median, standard deviation, minimum, and maximum for continuous data. T-tests were used to compare different time points and different dosages for the INF-γ and chemokine data. Statistical tests were conducted using SAS version 9.4.

RESULTS

Patient Characteristics

Nine male patients began study drug treatment at a median of 133 days (range, 70 to 175 days) after PBSCT. The median age of patients enrolled on study was 58 years (Table 1). All patients had autologous peripheral blood stem cells collected by apheresis following mobilization with filgrastim with (n = 6) or without (n = 3) plerixafor and received high-dose carmustine/ etoposide/ cytarabine/ melphalan (BEAM) as the preparative regimen prior to PBSCT. Supportive care after PBSCT was as previously described (48), except that patients did not routinely receive filgrastim support post-transplant.

Seven patients enrolled on study had DLBCL, including 2 patients with primary refractory disease and 5 patients with relapsed disease. Biopsy at the time of relapse or disease progression preceding PBSCT confirmed DLBCL for all 5 patients with recurrent disease and one of the 2 patients with primary refractory disease. One patient with DLBCL had biopsy-proven primary refractory disease after R-CHOP therapy and experienced rapid disease progression after one cycle of platinum-based salvage chemotherapy. This patient underwent PBSCT after responding to local radiation therapy. One patient had advanced stage mantle cell lymphoma in first complete response at the time of PBSCT. One patient with advanced stage indolent follicular experienced clinically suspected (not biopsy proven) transformation to aggressive lymphoma and underwent PBSCT after achieving a second complete response following salvage chemotherapy.

Administration of Ofatumumab and rhIL-18 on Study

Three patients each were enrolled in the 3, 10, and 30 μg/kg dose cohorts, respectively. All 9 patients received the 4 planned infusions of ofatumumab. Six patients received the 8 planned

infusions of rhIL-18. One patient with mantle cell lymphoma was taken of study for disease progression after receiving 6 of 8 planned doses (at 3 μg/kg) of rhIL-18. One patient with DLBCL received 6 of 8 planned doses (at 3 μg/kg) of rhIL-18 due to occurrence of asymptomatic grade 3 neutropenia (absolute neutrophil count 525 per μL) during week 7 of study. His absolute neutrophil count recovered to 3,800 within 14 days. It was retrospectively judged that this transient decline in neutrophils was likely due to delayed-onset neutropenia from recent ofatumumab infusions rather than due to rhIL-18. One patient with DLBCL received 6 of 8 planned doses (at 30 μg/kg) of rhIL-18 before being taken off study for disease progression). None of the enrolled patients was taken off study because of serious adverse events or failure to tolerate the treatment.

Clinical Toxicity and Laboratory Abnormalities during Administration of Ofatumumab and rhIL-18

Clinical adverse events attributed to rhIL-18 are summarized in Table 2. The only grade 3–4 non-hematologic laboratory abnormality observed during the study was grade 4 hypomagnesemia that was present at baseline in one patient (Table 3). This was attributed to prior chemotherapy. Hematologic toxicity (Table 4) was generally similar to that seen in patients receiving rhIL-18 as monotherapy by the same schedule of administration (17). Grade 1 adverse events attributed to ofatumumab included fever, chills, flu-like symptoms, flushing, headache, nausea, vomiting, diarrhea, and rash (one occurrence of each event). One patient had grade 2 fatigue attributed to ofatumumab. Grade 1–2 adverse events attributed to ofatumumab were not more common in the higher rhIL-18 dose cohorts as compared to the 3 μg/kg rhIL-18 dose cohort (data not shown). No grade 3 or 4 symptomatic adverse events were attributed to rhIL-18 or ofatumumab

Transient asymptomatic grade 3 neutropenia was seen during week 7 of study in two patients, one each in the 3 and 30 μg/kg cohorts; this was ultimately attributed to prior ofatumumab infusions and not to rhIL-18. Transient grade 3–4 lymphopenia was considered an expected biologic effect of rhIL-18 administration rather than an adverse event. No serious adverse events were attributed to ofatumumab or rhIL-18 in this study and a maximum tolerated dose of rhIL-18 was not identified.

Effects of rhIL-18 on Peripheral Blood Lymphocytes

The median absolute lymphocyte count of study patients at the time of the first ofatumumab infusion was 1,410 cells per μL, which is within the normal range (1,000 – 3,200 cells per μL) for our clinical laboratory. However, three patients (subjects 03, 05, and 09) had subnormal lymphocyte counts of 800, 500, and 700 cells per μl, respectively, when they commenced study drug treatment. The median absolute peripheral blood NK cell count was 220 cells per μl (range, 84 – 714 cells per μl).

Circulating lymphocytes declined and recovered in a manner similar to that seen in previous studies of rhIL-18 monotherapy (16, 17) or rhIL-18 plus rituximab (18). The mean reduction from pre-dose levels in the numbers of peripheral blood lymphocytes 4 hours after the first rhIL-18 infusion was 66%, 91%, and 81% for the 3, 10, and 30 μg/kg cohorts, respectively. Thus, the maximal reduction in circulating lymphocytes was seen in the 10 μg/kg dose

cohort. The mean reduction from pre-dose levels in the number of peripheral blood NK cells 4 hours after the first rhIL-18 infusion was 91%, 96%, and 97% for the 3, 10, and 30 μg/kg cohorts, respectively. Therefore, per the protocol-specified NK cell biomarker criteria enrollment of a 100 μg/kg dose cohort was not undertaken.

Effects of rhIL-18 on serum levels of IFN-γ and chemokines

Prior to the first infusion of rhIL-18, IFN-γ was undetectable (< 8 pg/mL) in serum samples obtained from 8 subjects. Subject 07 had a baseline serum IFN-γ level of 9 pg/mL. IFN-γ became detectable in serum samples obtained from 8 subjects at 4 and/or 6 hours after the first rhIL-18 infusion. Only subject 03 did not have detectable serum IFN-γ levels during rhIL-18 treatment. The peak serum IFN-γ levels seen after the first rhIL-18 infusion were: 11, 14, and 1 pg/mL for the 3 subjects in the 3 μg/kg cohort; 21, 9, and 14 pg/mL for the 3 subjects in the 10 μg/kg cohort; and 57, 76, and 62 pg/mL for the 3 subjects in the 30 μg/kg cohort. The mean serum IFN-γ levels were not significantly different (P = 0.3632) when comparing samples obtained 4 hours after the first rhIL-18 infusion from subjects in the 3 versus 10 μg/kg dose cohorts. The mean serum IFN-γ levels seen 4 hours after the first rhIL-18 infusion were significantly higher in samples obtained from subjects in the 30 μg/kg dose cohort compared to the levels seen in the 3 μg/kg (P = 0.0019) and 10 μg/kg (P = 0.0035) dose cohorts. Similar results were observed for samples obtained 6 hours after the first rhIL-18 infusion (data not shown).

The median serum MIG (CXCL9) level prior to study drug treatment was 146 pg/mL (range, 67 – 785 pg/mL). Three subjects had baseline MIG levels that exceeded the upper limit value (199 pg/mL) seen in healthy control volunteers (data provided by manufacturer of the ELISA assay). Peak serum MIG levels increased from baseline values by a median of 3.2-fold (range, 0.9 – 10.9-fold) in samples obtained 4 or 6 hours after the first rhIL-18 infusion. Peak serum MIG levels increased by a median of 1.2-fold (range, 0.9 – 3.3-fold) in the 3 μg/kg cohort, 3.9-fold (range, 1.5 – 5.4-fold) in the 10 μg/kg cohort, and 3.2-fold (range, 3.0 – 10.9-fold) in the 30 μg/kg cohort.

The median baseline serum IP-10 (CXCL10) level prior to study drug treatment was 207 pg/mL (range, 67 – 359 pg/mL), which is within the range (38 – 361 pg/mL) seen in healthy control volunteers (data provided by manufacturer of the ELISA assay). Serum IP-10 levels increased to values exceeding 500 pg/mL (the upper limit of linearity of the ELISA assay) in samples obtained from all subjects 4 hours after the first rhIL-18 infusion and remained significantly elevated in samples obtained 6 hours post-dose (Table 5). Serum IP-10 levels 6 days after the first rhIL-18 infusion (on Week 2, Day 1 of study) remained modestly higher than baseline levels, but this was statistically significant only for subjects in the 10 μg/kg cohort (Table 5).

Progression-Free Survival of Study Patients

One patient with mantle cell lymphoma and one patient with DLBCL progressed while receiving rhIL-18 and were taken off study. The other 7 patients with aggressive lymphoma (6 DLBCL, 1 presumed transformed lymphoma) are alive and free of lymphoma progression with a median follow-up of 41.6+ months (range, 28.6+ to 55.1+ months) after PBSCT.

DISCUSSION

This phase I study clearly shows that combined therapy with rhIL-18 and ofatumumab is feasible after PBSCT for lymphoma. Adverse effects were modest and tolerable and no dose-limiting toxicities were observed. Asymptomatic, transient grade 4 neutropenia occurred in two subjects. These episodes of neutropenia were attributed to ofatumumab and were not associated with fever or infection. A maximum tolerated dose of rhIL-18 has not been identified in clinical trials to date. Escalation to a 100 μg/kg dose cohort of rhIL-18 was not undertaken in our study based on a prospectively planned NK cell biomarker analysis. Given the modest toxicity of rhIL-18 and ofatumumab after PBSCT, combination with additional immunotherapeutic agents should be feasible. Rational choices could include adding lirilumab to further augment NK cell ADCC against antibody-sensitized lymphoma cells (49) and/or adding an immune checkpoint inhibitor (e.g. ipilimumab or anti-PD1 antibodies) to enhance T cell-mediated antitumor immunity (50, 51).

IFN-γ levels increased in serum of all but one patient given rhIL-18 on this study, consistent with our hypothesis that STAT4-deficient lymphocytes after PBSCT would be responsive to stimulation with IL-18. IFN-γ can promote the differentiation of Th1 helper effector cells (52). IFN-γ secreted by activated NK cells also stimulates production of the CXC chemokines MIG (CXCL9) and IP-10 (CXCL10), which contribute to antitumor responses by inhibiting tumor angiogenesis and recruiting CXCR3-bearing effector cells (53–55). However, IFN-γ induces the expression of indoleamine 2,3-dioxygenase (IDO1) in tumor cells, antigen-presenting cells, myeloid-derived suppressor cells, and immune effector cells (51, 56). IDO1 is an enzyme that catalyzes the breakdown of the essential amino acid tryptophan. IDO1-mediated tryptophan depletion suppresses effector T cell responses (51, 56). Moreover, IDO1 activity yields immunosuppressive metabolites, such as kynurenine and quinolinic acid. These metabolites inhibit the proliferation and cytotoxic function of T and NK cells (51, 56). IFN-γ can also induce the expression of PD-L1 on tumor cells (57). Therefore, administration of IDO1 inhibitors may be necessary to circumvent IFN-γ-mediated feedback inhibition of T and NK cells during post-transplant immunotherapy. Several oral inhibitors of IDO1 are in clinical development (58).

The efficacy of rhIL-18 and ofatumumab after PBSCT cannot be accurately assessed in this phase I clinical trial. Nevertheless, the seven patients who were not taken off study early for progressive disease remain alive without evidence of active lymphoma at a median of 3.5 years post-transplant. Indeed, all of these patients have remained progression free for more than 2.4 years post- PBSCT. Historical data indicate that about 3 to 4 of these patients with primary refractory or relapsed aggressive lymphoma would have been expected to develop disease progression by two years post-transplant. These promising results support continued efforts to develop novel approaches to immunotherapy after PBSCT for lymphoma.

Acknowledgments

Source of Funding: This study was supported in part by NIH grants UL RR025761 (Indiana Clinical and Translational Sciences Institute) and P30 CA82709 (Indiana University Simon Cancer Center). Additional support was provided by the Indiana University Health-Indiana University School of Medicine Strategic Research Initiative. Clinical trial design was based in part on preclinical studies supported by NIH grant RO1 CA118118 (MJR). The spouse of MJR was an employee of and has equity in Eli Lilly and Company.

The authors wish to thank the nurses in the Indiana Clinical Research Center for support in the completion of this clinical trial, Sandra Althouse for performing statistical analyses, and Lijun Ni for assistance with the correlative laboratory studies.

References

1. Aisenberg AC. Coherent view of non-Hodgkin's lymphoma. J Clin Oncol. 1995; 13:2656–75. [PubMed: 7595720]
2. Philip T, Guglielmi C, Hagenbeek A, Somers R, Lelie Hvd, Bron D, Sonneveld P, Gisselbrecht C, Cahn J-Y, Harousseau J-L, Coiffier B, Biron P, Mandelli F, Chauvin F. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995; 333:1540–5. [PubMed: 7477169]
3. Vose JM, Rizzo DJ, Tao-Wu J, Armitage JO, Bashey A, Burns LJ, Christiansen NP, Freytes CO, Gale RP, Gibson J, Giralt SA, Herzig RH, Lemaistre CF, McCarthy PL, Nimer SD, Petersen FB, Schenkein DP, Wiernik PH, Wiley JM, Loberiza FR, Lazarus HM, Van Besien K, Horowitz MM. Autologous transplantation for diffuse aggressive non-Hodgkin lymphoma in first relapse or second remission. Biol Blood Marrow Transplant. 2004; 10:116–27. [PubMed: 14750077]
4. Yuen AR, Rosenberg SA, Hoppe RT, Halpern JD, Horning SJ. Comparison between conventional salvage therapy and high-dose therapy with autografting for recurrent or refractory Hodgkin's disease. Blood. 1997; 89:814–22. [PubMed: 9028312]
5. Ferme C, Mounier N, Divine M, Brice P, Stamatoullas A, Reman O, Voillat L, Jaubert J, Lederlin P, Colin P, Berger F, Salles G. Intensive salvage therapy with high-dose chemotherapy for patients with advanced Hodgkin's disease in relapse or failure after initial chemotherapy: results of the Groupe d'Etudes des Lymphomes de l'Adulte H89 trial. J Clin Oncol. 2002; 20:467–75. [PubMed: 11786576]
6. Klingemann H-G, Phillips GL. Immunotherapy after bone marrow transplantation. Bone Marrow Transplant. 1991; 8:73–81. [PubMed: 1718516]
7. Robertson MJ, Pelloso D, Abonour R, Hromas RA, RPN, Wood L, Cornetta K. Interleukin-12 immunotherapy after autologous stem cell transplantation for hematologic malignancies. Clin Cancer Res. 2002; 8:3383–93. [PubMed: 12429625]
8. Pelloso D, Cyran K, Timmons L, Williams BT, Robertson MJ. Immunological consequences of interleukin 12 administration after autologous stem cell transplantation. Clin Cancer Res. 2004; 10:1935–42. [PubMed: 15041709]
9. Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, Schreiber RD. Demonstration of an interferon-γ-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci USA. 1998; 95:7556–61. [PubMed: 9636188]
10. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFN-γ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature. 2001; 410:1107–11. [PubMed: 11323675]
11. Street SEA, Trapani JA, MacGregor D, Smyth MJ. Suppression of lymphoma and epithelial malignancies by inteferon γ. J Exp Med. 2002; 196:129–34. [PubMed: 12093877]
12. Leibson P. Signal transduction during natural killer cell activation: inside the mind of a killer. Immunity. 1997; 6:655–61. [PubMed: 9208838]
13. Trotta R, Dal Col J, Yu J, Ciarlariello D, Thomas B, Zhang X, Allard J, Wei M, Mao H, Byrd JC, Perrotti D, Caligiuri MA. TGF-β utilizes SMAD3 to inhibit CD16-mediated IFN-γ production and antibody-dependent cellular cytotoxicity in human NK cells. J Immunol. 2008; 181:3784–92. [PubMed: 18768831]
14. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Ann Rev Immunol. 2001; 19:423–74. [PubMed: 11244043]
15. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. J Leukoc Biol. 2003; 73:213–24. [PubMed: 12554798]
16. Robertson MJ, Mier JW, Logan T, Atkins M, Koon H, Koch KM, Kathman S, Pandite LN, Oei C, Kirby LC, Jewell RC, Bell WN, Thurmond LM, Weisenbach J, Roberts S, Dar MM. Clinical and biological effects of recombinant human interleukin-18 administered by intravenous infusion to patients with advanced cancer. Clin Cancer Res. 2006; 12:4265–73. [PubMed: 16857801]

17. Robertson MJ, Kirkwood JM, Logan TF, Koch KM, Kathman S, Kirby LC, Bell WN, Thurmond LM, Weisenbach J, Dar MM. A dose-escalation study of recombinant human interleukin-18 using two different schedules of administration in patients with cancer. Clin Cancer Res. 2008; 14:3462–9. [PubMed: 18519778]

18. Robertson MJ, Kline J, Struemper H, Koch KM, Bauman JW, Gardner OS, Murray SC, Germaschewski F, Weisenbach J, Jonak Z, Toso JF. A dose-escalation study of recombinant human interleukin-18 in combination with rituximab in patients with non-Hodgkin lymphoma. J Immunother. 2013; 36:331–41. [PubMed: 23799412]

19. Ansell SM, Witzig TE, Kurtin PJ, Sloan JA, Jelinek DF, Howell KG, Markovic SN, Habermann TM, Klee GG, Atherton PJ, Erlichman C. Phase 1 study of interleukin-12 in combination with rituximab in patients with B-cell non-Hodgkin lymphoma. Blood. 2002; 99:67–74. [PubMed: 11756154]

20. Cartron G, Zhao-Yang L, Baudard M, Kanouni T, Rouille V, Quittet P, Klein B, Rossi J-F. Granulocyte-macrophage colony-stimulating factor potentiates rituximab in patients with relapsed follicular lymphoma: results of a phase II study. J Clin Oncol. 2008; 26:2725–31. [PubMed: 18427151]

21. Friedberg JW, Neuberg D, Gribben JG, Fisher DC, Canning C, Koval M, Poor CM, Green LM, Daley J, Soiffer R, Ritz J, Freedman AS. Combination immunotherapy with rituximab and interleukin 2 in patients with relapsed or refractory follicular non-Hodgkin's lymphoma. Br J Haematol. 2002; 117:828–34. [PubMed: 12060117]

22. Gluck WL, Hurst D, Yuen A, Levine AM, Dayton MA, Gockerman JP, Lucas J, Denis-Mize K, Tong B, Navis D, Difrancesco A, Milan S, Wilson SE, MW. Phase I studies of interleukin (IL)-2 and rituximab in B-cell non-Hodgkin's lymphoma: IL-2 mediated natural killer cell expansion correlates with clinical response. Clin Cancer Res. 2004; 10:2253–64. [PubMed: 15073100]

23. Khan KD, Emmanouilides C, Benson DM, Hurst D, Garcia P, Michelson G, Milan S, Ferketich AK, Piro L, Leonard JP, Porcu P, Eisenbeis CF, Banks AL, Chen L, Byrd JC, Caligiuri MA. A phase 2 study of rituximab in combination with recombinant interleukin-2 for rituximab-refactory indolent non-Hogkin's lymphoma. Clin Cancer Res. 2006; 2006:7046–53.

24. Robertson MJ, Soiffer RJ, Wolf SF, Manley TJ, Donahue C, Young D, Herrmann SH, Ritz J. Response of human natural killer (NK) cells to NK cell stimulatory factor (NKSF): cytolytic activity and proliferation of NK cells is differentially regulated by NKSF. J Exp Med. 1992; 175:779–88. [PubMed: 1346796]

25. Liu Z, Lee F-T, Hanai N, Smyth FE, Burgess AW, Old LJ, Scott AM. Cytokine enhancement of in vitro antibody-dependent cellular cytotoxicity mediated by chimeric anti-GD3 monoclonal antibody KM871. Cancer Immunity. 2002; 2:13–27. [PubMed: 12747758]

26. Eisenbeis CF, Fischer B, Baiocchi RA, Carrodeguas L, SR, Chen L, Banks AL, Davis D, Young D, Kelbick N, Stephens J, Byrd JC, Grever MR, Caligiuri MA, Porcu P. Combination immunotherapy of B-cell non-Hodgkin's lymphoma with rituximab and interleukin-2: a preclinical and phase I study. Clin Cancer Res. 2004; 10:6101–10. [PubMed: 15447996]

27. Connor RI, Shen L, Fanger MW. Evaluation of the antibody-dependent cytotoxic capabilites of individual human monocytes: role of FcγRI and FcγRII and the effects of cytokines at the single cell level. J Immunol. 1990; 145:1483–9. [PubMed: 1696598]

28. Young DA, Lowe LD, Clark SC. Comparison of the effects of IL-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor in supporting monocyte differentiation in culture: analysis of macrophage antibody-dependent cellular cytotoxicity. J Immunol. 1990; 145:607–15. [PubMed: 2142182]

29. Logan TF, Robertson MJ. Interleukins 18 and 21: biology, mechanisms of action, toxicity, and clinical activity. Curr Oncol Reports. 2006; 8:114–9.

30. Casares N, Arribillaga L, Sarobe P, Dotor J, Lopez Diaz de Cerio A, Melero I, Prieto J, Borras-Cuesta F, Lasarte JJ. CD4+/CD25+ regulatory cells inhibit activation of tumor-primed CD4+ T cells with IFN-γ-dependent antiangiogenic activity, as well as long-lasting tumor immunity elicited by peptide vaccination. J Immunol. 2003; 171:5931–9. [PubMed: 14634104]

31. Ahmadzadeh M, Rosenberg SA. IL-2 administration increases CD4+ CD25^{hi} Foxp3+ regulatory T cells in cancer patients. Blood. 2006; 107:2409–14. [PubMed: 16304057]

32. Shah MH, Freud AG, Benson DM, Ferkitich AK, Dezube BJ, Bernstein ZP, Caligiuri MA. A phase I study of ultra low dose interleukin-2 and stem cell factor in patients with HIV infection or HIV and cancer. Clin Cancer Res. 2006; 12:3993–6. [PubMed: 16818697]
33. Chang H-C, Han L, Goswami R, Nguyen ET, Pelloso D, Robertson MJ, Kaplan MH. Impaired development of human Th1 cells in patients with deficient expression of STAT4. Blood. 2009; 113:5887–90. [PubMed: 19359411]
34. Lupov IP, Voiles L, Han L, Schwartz A, De La Rosa M, Oza K, Pelloso D, Sahu RP, Travers JB, Robertson MJ, Chang H-C. Acquired STAT4 deficiency as a consequence of cancer chemotherapy. Blood. 2011; 118:6097–106. [PubMed: 21998209]
35. Robertson MJ, Chang H-C, Pelloso D, Kaplan MH. Impaired interferon-γ production as a consequence of STAT4 deficiency after autologous hematopoietic stem cell transplantation for lymphoma. Blood. 2005; 106:963–70. [PubMed: 15817683]
36. Cheson BD. Ofatumumab, a novel anti-CD20 monoclonal antibody for the treatment of B-cell malignancies. J Clin Oncol. 2010; 28:3525–30. [PubMed: 20458041]
37. Hagenbeek A, Gadeberg O, Johnson P, Pedersen LM, Walewski J, Hellmann A, Link BK, Robak T, Wojtukiewicz M, Pfreundschuh M, Kneba M, Engert A, Sonneveld P, Flensburg M, Petersen J, Losic N, Radford J. First clinical use of ofatumumab, a novel fully human anti-CD20 monoclonal antibody in relapsed or refractory follicular lymphoma: results of a phase 1/2 trial. Blood. 2008; 111:5486–95. [PubMed: 18390837]
38. Teeling JL, Mackus WJM, Wiegman LJJM, van den Brakel JHN, Beers SA, French RR, van Meerten T, Ebeling S, Vink T, Slootstra JW, Parren PWHI, Glennie MJ, van de Winkel JGJ. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol. 2006; 177:362–71. [PubMed: 16785532]
39. Teeling JL, French RR, Cragg MS, van den Brakel JHN, Pluyter M, Huang H, Chan C, Parren PWHI, Hack CE, Dechant M, Valerius T, van de Winkel JGJ, Glennie MJ. Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. Blood. 2004; 104:1793–800. [PubMed: 15172969]
40. Li B, Zhao L, Wang C, Zhang X, Wu L, Chen L, Tong Q, Qian W, Wang H, Guo Y. Characterization of a rituximab variant with potent antitumor activity against rituximab-resistant B-cell lymphoma. Blood. 2009; 114:5007–15. [PubMed: 19828699]
41. Craigen JL, Mackus WJM, Engleberts P, Miller SR, Speller S, Chamberlain LC, Davis BG, McHugh SM, Bullmore E, Cox CJ, Wetten S, Perdock G, Bakker JM, van de Winkel JGJ, Parren PWHI. Ofatumumab, a human monoclonal antibody targeting a membrane-proximal small-loop epitope on CD20, induces potent natural killer cell-mediated antibody-dependent cytotoxicity. Blood. 2009; 114:687. (abstract 1725).
42. Barth MJ, Mavis C, Czuczman MS, hernandez-ilizaliturri FJ. Ofatumumab exhibits enhanced *in vitro* and *in vivo* activity compared to rituximab in preclinical models of mantle cell lymphoma. Clin Cancer Res. 2015; 21:4391–7. [PubMed: 25964296]
43. Lotze MT, Matory YL, Ettinghausen SE, Rayner AA, Sharrow SO, Seipp CAY, Custer MC, Rosenberg SA. In vivo administration of purified human interleukin 2: II. Half-life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL 2. J Immunol. 1985; 135:2865–75. [PubMed: 2993418]
44. Robertson MJ, Cameron C, Atkins MB, Gordon MS, Lotze MT, Sherman ML, Ritz J. Immunologic effects of interleukin 12 administered by bolus intravenous injection to patients with cancer. Clin Cancer Res. 1999; 5:9–16. [PubMed: 9918197]
45. Robertson MJ, Caligiuri MA, Manley TJ, Levine H, Ritz J. Human natural killer cell adhesion molecules: differential expression after activation and participation in cytolysis. J Immunol. 1990; 145:3194–201. [PubMed: 1700001]
46. Swerdlow, SH., Campo, E., Harris, NL., Jaffe, ES., Pileri, SA., Stein, H., Thiele, J., Vardiman, JW. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2008.
47. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, Lister TA, Vose J, Grillo-Lopez A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris NL, Armitage JO, Carter W, Hoppe R, Canellos GP. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. J Clin Oncol. 1999; 17:1244–53. [PubMed: 10561185]

48. Robertson MJ, Abonour R, Hromas R, Nelson RP, Fineberg NS, Cornetta K. Augmented high-dose regimen of cyclophosphamide, carmustine, and etoposide with autologous hematopoietic stem cell transplantation for relapsed and refractory aggressive non-Hodgkin's lymphoma. Leuk Lymphoma. 2005; 46:1477–87. [PubMed: 16194894]

49. Kohrt HE, Thielens A, Marabelle A, Sagiv-Barfi I, Sola C, Chanuc F, Fuseri N, Bonnafous C, Czerwinski D, Rajapaksa A, Waller E, Ugolini S, Vivier E, Romagne F, Levy R, Blery M, Andre P. Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherpy and in combination with anti-CD20 antibodies. Blood. 2014; 123:678–86. [PubMed: 24326534]

50. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol. 2015; 33:1974–82. [PubMed: 25605845]

51. Andersen MH. The targeting of immunosuppressive mechanisms in hematological malignancies. Leukemia. 2014; 28:1784–92. [PubMed: 24691076]

52. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-γ: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004; 75:163–89. [PubMed: 14525967]

53. Coughlin CM, Salhany KE, Gee MS, LaTemple DC, Kotenko S, Ma X, Gri G, Wysocka M, Kim JE, Liu L, Laio F, Farber JM, Pestka S, Trinchieri G, Lee WMF. Tumor cell responses to IFN-γ affect tumorigenicity and response to IL-12 therapy and antiangiogenesis. Immunity. 1998; 9:25–34. [PubMed: 9697833]

54. Robertson MJ. Role of chemokines in the biology of natural killer cells. J Leukoc Biol. 2002; 71:173–83. [PubMed: 11818437]

55. Tannenbaum CS, Tubbs R, Armstrong D, Finke J, Bukowski RM, Hamilton TA. The CXC chemokines IP-10 and Mig are necessary for IL-12-mediated regression of the mouse RENCA tumor. J Immunol. 1998; 161:927–32. [PubMed: 9670971]

56. Prendergast GC, Smith C, Thomas S, Mandik-Nayak L, Laury-Kleintop L, Metz R, Muller AJ. Indoleamine 2, 3-dioxygenase pathways of inflammation and immune escape in cancer. Cancer Immunol Immunother. 2014; 2–14:721–35.

57. Mandai M, Hamanishi J, Abiko K, Matsumara N, Baba T, Konishi T. Dual faces of IFN-γ in cancer progression: a role of PD-L1 induction in the determination of pro- and antitumor immunity. Clin Cancer Res. 2016:22.

58. Sheridan C. IDO inhibitors move center stage in immuno-oncology. Nature Biotech. 2015; 33:321–2.

TABLE 1

Characteristics of Study Patients

Age (y), median (range)	58 (42–69)
Histologic subtype of lymphoma	
DLBCL	7
Follicular lymphoma, grade 2 *	1
Mantle cell lymphoma	1
Prior rituximab treatment	
With induction chemotherapy	9
With salvage therapy prior to PBSCT	6
Disease status after salvage therapy (prior to PBSCT)	
First complete remission	1
Chemosensitive primary refractory disease	1
Chemorefractory primary refractory disease	1
Chemosensitive relapsed disease	6
Complete response	6
Partial response	3
Disease status at time of study enrollment (after PBSCT)	
Complete response	7
Partial response	2

* Clinically suspected transformation to aggressive lymphoma

TABLE 2

Clinical Adverse Events Attributed to rhIL-18

Adverse Event	rhIL-18 Doses plus Ofatumumab (1000 mg)			
	No. Patients with Grade 1–2 Adverse Event (%)*			
	3 μg/kg (N = 3)	10 μg/kg (N = 3)	30 μg/kg (N = 3)	Total (N = 9)
Fever	1 (33)	1 (33)	0	2 (67)
Chills	1 (33)	1 (33)	0	2 (67)
Fatigue	1 (33)	1 (33)	0	2 (67)
Flu-like sympoms	0	1 (33)	0	1 (33)
Nausea	0	1 (33)	0	1 (33)
Anorexia	1 (33)	0	0	1 (33)
Constipation	1 (33)	0	0	1 (33)
Diarrhea	0	0	1 (33)	1 (33)
Abdominal distension	1 (33)	0	0	1 (33)
Abdominal pain	1 (33)	0	0	1 (33)
Back pain	0	1 (33)	0	1 (33)
Headache	1 (33)	0	1 (33)	2 (67)
Unspecified pain	1 (33)	0	0	1 (33)
Skin rash	0	1 (33)	0	1 (33)

*No grade 3 or 4 adverse events were attributed to rhIL-18.

TABLE 3

Non-hematologic Laboratory Abnormalities (Regardless of Causality)

	rhIL-18 Doses plus Ofatumumab (1000 mg)			
	No. Patients with Grade 1–2 / 3–4 Laboratory Abnormality			
Laboratory Abnormality	**3 μg/kg (N = 3)**	**10 μg/kg (N = 3)**	**30 μg/kg (N = 3)**	**Total (N = 9)**
Elevated Bilirubin	0 / 0	1 / 0	0 / 0	1 / 0
Elevated Alkaline Phosphatase	2 / 0	0 / 0	1 / 0	3 / 0
Elevated SGOT	0 / 0	1 / 0	0 / 0	1 / 0
Elevated Creatinine	0 / 0	0 / 0	1 / 0	1 / 0
Hyperglycemia	3 / 0	2 / 0	3 / 0	8 / 0
Hypomagnesemia	0 / 0	0 / 0	0 / 1*	0 / 1*
Hyponatremia	1 / 0	0 / 0	1 / 0	2 / 0
Hypocalcemia	1 / 0	0 / 0	0 / 0	1 / 0
Hypokalemia	1 / 0	1 / 0	0 / 0	2 / 0
Hypoalbuminemia	1 / 0	0 / 0	1 / 0	2 / 0

*Asymptomatic grade 4 hypomagnesemia was present at baseline prior to study drug administration.

TABLE 4

Hematologic Abnormalities (Regardless of Causality)

	rhIL-18 Doses plus Ofatumumab (1000 mg)			
	No. Patients with Grade 1–2 / 3* Hematologic Abnormality			
Reduction in Level of	**3 μg /kg (N = 3)**	**10 μg /kg (N = 3)**	**30 μg /kg (N = 3)**	**Total (N = 9)**
Hemoglobin	3 / 0	3 / 0	1 / 1**	7 / 1**
Platelet count	2 / 0	2 / 0	3 / 0	7@ / 0
Neutrophil count	0 / 1***	1 / 0	2 / 1***	3 / 2***

*No grade 4 hematologic abnormalities were seen.

**Baseline grade 2 anemia increased to grade 3 during study. This increase in toxicity grade was attributed to phlebotomy and not to study drugs.

@All grade 1; no grade 2 thrombocytopenia was seen.

***Transient asymptomatic grade 3 neutropenia in two patients was attributed to prior ofatumumab infusions.

TABLE 5

Serum IP-10 Levels after first infusion of rhIL-18

rhIL-18 Dose Cohort	Serum IP-10 Levels (pg/mL; mean ± SD) (P values from t-test comparing post-dose to baseline values)			
	Baseline (Pre-dose)	**4 hours after rhIL-18 dose**	**6 hours after rhIL-18 dose**	**Week 2, Day 1**
3 μg/kg (N = 3)	145 ± 59	500 ± 0 (P = 0.0090)	468 ± 55 (P = 0.0022)	203 ± 51 (P = 0.2675)
10 μg/kg (N = 3)	94 ± 54	500 ± 0 (P = 0.0057)	500 ± 0 (P = 0.0057)	188 ± 23 (P = 0.0499)
30 μg/kg (N = 3)	110 ± 45	500 ± 0 (P = 0.0044)	500 ± 0 (P = 0.0044)	142 ± 27 (P = 0.3544)